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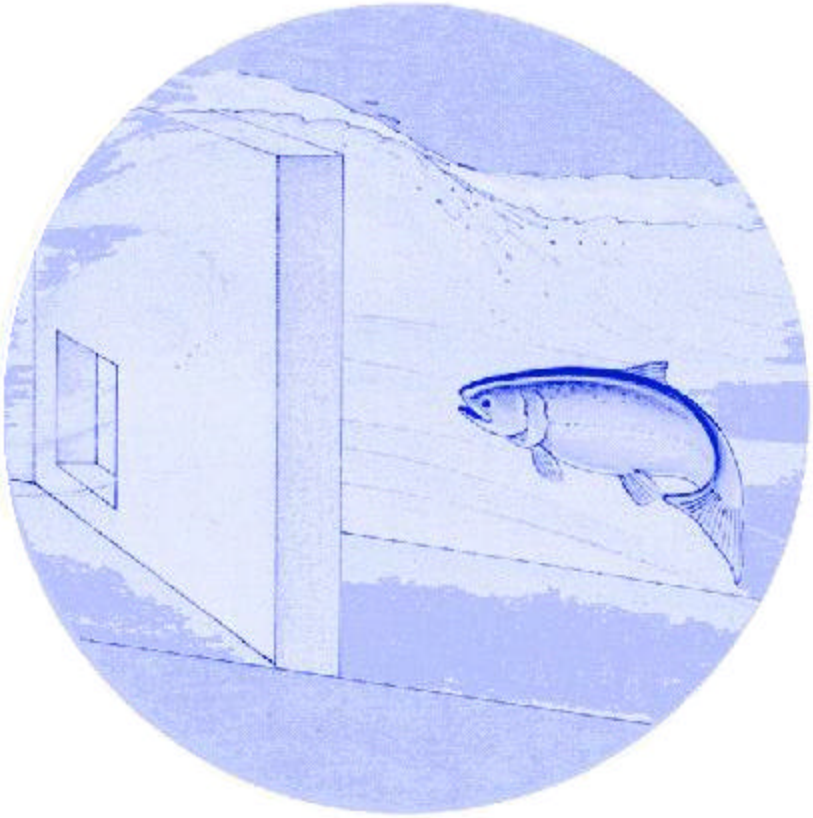
Progress Report
2001 - 2002



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Influences of Stocking Salmon Carcass Analogs on Salmonids in Yakima River Tributaries

Progress Report - May 2001- December 2002

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Executive Summary

The benefits that marine derived nutrients from adult salmon carcasses provide to juvenile salmonids are increasingly being recognized. Current estimates suggest that only 6-7% of marine-derived nitrogen and phosphorous that were historically available to salmonids in the Pacific Northwest are currently available. Food limitation may be a major constraint limiting the restoration of salmonids. A variety of methods have been proposed to offset this nutrient deficit including: allowing greater salmon spawning escapement, stocking hatchery salmon carcasses, and stocking inorganic nutrients. Unfortunately, each of these methods has some ecological or socio-economic shortcoming. We intend to overcome many of these shortcomings by making and evaluating a pathogen free product that simulates a salmon carcass (analog).

Abundant sources of marine derived nutrients are available such as fish offal from commercial fishing and salmon carcasses from hatcheries. However, a method for recycling these nutrients into a pathogen free analog that degrades at a similar rate as a natural salmon carcass has never been developed. We endeavored to 1) develop a salmon carcass analog that will increase the food available to salmonids, 2) determine the pathways that salmonids use to acquire food from analogs, and 3) determine the benefits to salmonids and the potential for application to salmonid restoration. We used a before-after-control-impact-paired design in six tributaries of the upper Yakima basin to determine the utility of stocking carcass analogs.

Our preliminary results suggest that the introduction of carcass analogs into food-limited streams can be used to restore food pathways previously provided by anadromous salmon. The analogs probably reproduced both of the major food pathways that salmon carcasses produce: direct consumption and food chain enhancement. Trout and salmon fed directly on the carcass analogs during the late summer and presumably benefited from the increased invertebrate biomass later in the year. Future reports will analyze whether any benefits are statistically detectable. The risks of using carcass analogs also appear to be low. Pathogens appear to be killed in the manufacturing process of the analogs. In addition, preliminary results suggest that fish exposed to the analogs did not have higher incidences of pathogens. The water quality was also not degraded by the analog additions with the exception of a temporary surface film. Finally, our anecdotal observations, suggested that there was not an increase in the number of predators during the first year of analog distribution. In summary, the risks of analog placement appear to be low but the benefits appear to be high.

All results should be considered preliminary until further analyses and field work are conducted.

Introduction

Salmonid populations that rear in tributaries of the Columbia River appear to have relatively low food availability, which may be contributing to reduced growth and survival, and ultimately hindering restoration efforts. Historically, large numbers of salmon returned to natal rivers to spawn (Gresh et al. 2000). These salmon contributed huge amounts of nutrients to aquatic ecosystems via their carcasses and eggs (Larkin and Slaney 1997; Gresh et al. 2000). Gresh et al. (2000) estimated that only 6-7% of the marine-derived nitrogen and phosphorous historically delivered to rivers of the Pacific Northwest is currently reaching those streams. Salmon eggs and carcasses are eaten by invertebrates and fish and the nutrients released by the decomposing carcasses can facilitate increased plant and microbial production that subsequently increases invertebrate production resulting in increased food availability for fish (Bilby et al. 1996). Unfortunately, the numbers of adult salmon that spawn in streams has been severely reduced (Nehlsen et al. 1991). Undoubtedly this has caused a reduction in the availability of food for young salmon and trout. For example, average stomach fullness of salmon and trout in the Yakima basin rarely exceeded 30% during the summer and fall of 1998 and 1999 when densities of salmon were very low (James et al. 1999, Pearsons, unpublished data). When the availability of food (freeze dried krill) was experimentally increased in the Yakima and John Day basins, salmonids readily increased their feeding activity until satiated (Pearsons, unpublished data). In addition to a reduction in the amount of marine derived nutrients, the capacity of stream systems to retain nutrients has also been diminished because of reduction in stream complexity and increases in peak flows (Pearsons et al. 1992). Recently, carcasses from hatchery salmon have been released into streams in an attempt to replace lost marine derived nutrients and associated productivity.

Stream fishes with access to salmon carcasses and eggs generally grow faster than fishes at locations without this material. Artificially increasing availability of salmon carcass flesh and eggs by adding carcasses of hatchery coho salmon *Oncorhynchus kisutsh* to a small stream in southwestern Washington doubled the growth rate of juvenile coho salmon at this site relative to a nearby stream reach with low availability of carcasses (Bilby et al. 1998). Rapid increases in the proportion of carcass-derived N (as indicated by nitrogen stable isotope values) in the muscle tissue of the juvenile fish at the treated site and the abundance of eggs and flesh in their stomachs clearly indicated that the material derived from the carcasses was responsible for the accelerated growth rate. The fish residing at the site to which carcasses had been added contained nearly 20 times the amount of material in their stomachs than did fish collected on the same date from an area without carcasses; 60% to 95% of it salmon eggs and flesh. Spawning salmon also have been shown to elevate growth rates of fish in Alaskan streams. In the Wood River watershed in southwest Alaska, char and trout grew very rapidly while carcasses of sockeye salmon *Oncorhynchus nerka* were present (Eastman 1996). The body weight of one tagged char increased 58% in 36 days.

Increases in the body size of juvenile salmonids can significantly increase their survival. Larger body size has been positively correlated with overwinter survival of juvenile coho salmon (Hartmann and Scrivner 1990; Quinn and Peterson 1996). Larger smolts enjoy a considerable advantage in survival once entering the marine environment (Bilton et al. 1982; Ward and Slaney 1988; Holtby et al. 1990; Tipping 1997). The effect of salmon carcass abundance on survival is

illustrated in the Skagit River, Washington, where the survival rate from fry to adult for coho salmon is positively related to the abundance of pink salmon *Oncorhynchus gorbuscha* spawning while the coho parr are rearing in the river (Michael 1995). The number of coho salmon recruits-per-spawner is about 1 during years of low availability of pink salmon carcasses but increases to over 3 during years of high carcass availability. Stocking hatchery salmon carcasses has great potential to restore wild salmonid populations, however the strategy is not without risk.

Potential ecological risks of stocking hatchery carcasses include, pathogen transfer, overabundance of nutrients, and increased predation. Stocking carcasses that have pathogens may increase the susceptibility of salmonids to a variety of diseases. Concerns about disease transmission have led Oregon and Washington to institute prohibitions on the transfer of carcasses out of the watershed of origin. As a result, there are many nutrient poor systems where placement of carcasses is not an option due to the absence of an approved source. The addition of salmon carcasses to mitigate for low nutrient levels is further limited by low carcass availability even in many watersheds with hatcheries. There often are not enough carcasses from hatcheries to produce nutrients comparable to what salmon historically contributed (Gresh et al. 2000).

Additional concerns have been raised by the practice of adding hatchery carcasses to streams. Stocking too many carcasses into an area may result in excessive nutrient supplies that could contribute to high levels of algae growth and cause poor water quality, such as low dissolved oxygen, or cause boom and bust food availability, which may negatively impact salmonid populations. However, this problem has never been observed with any of the carcass release experiments conducted to date (Bilby et al. 1998). Finally, carcasses may attract piscivorous predators that may feed on juvenile salmon or resident trout once the supply of carcasses has been depleted.

There are some alternative approaches to stocking hatchery carcasses, which may have lower ecological risks and more broad scale application (i.e., not enough hatchery carcasses to meet the need). One method that has been used widely in British Columbia is the addition of inorganic nutrients during the spring and fall (Johnston et al. 1990; Ashley and Slaney 1997). The nutrients stimulate algae growth, increase invertebrate production, and elevate food availability for the fish. However, this method does not directly provide a food source during the fall (e.g., fish flesh), for fish or wildlife, as spawning salmon do (Bilby et al. 1998). Another possible option is to stock pathogen free carcass analogs. The advantages of using carcass analogs, as opposed to hatchery salmon carcasses or commercial fertilizers, are 1) mimic natural pathways of food production, 2) pathogen free so they can be stocked in any suitable water, 3) abundance is potentially very large and independent of salmon runs, 4) easy to store, carry and distribute, 5) contains rare earth elements that may be important for salmonid survival, and 6) recycle nutrients from fish byproducts that would ordinarily be treated as waste. Analogs could be produced from unused fish parts from commercial fisheries and may provide the same nutrient and food benefits as salmon carcasses. The stocking of analogs could still produce excess nutrients if too many are placed at a location or increase the abundance of piscivorous predators at a placement site. However, the risk of disease should be eliminated.

In this report, we present preliminary qualitative information about the benefits and risks of stocking salmon carcass analogs into a variety of Yakima Basin tributaries containing resident and anadromous trout and salmon. The evaluation of benefits and risks can then be used to determine if the wide scale use of carcass analogs represents a useful fisheries enhancement tool. More specifically, in future reports we will test whether salmonid growth and abundance, fish

community composition, and biomass differed among areas with and without carcass analogs. We also present information on the pathways and processes by which the stream biota utilize analog material.

Methods

Before individual tasks are described, it is worthwhile to describe the overall design and philosophy of the proposed work. After the carcass analogs were developed (Appendix 1), they were distributed into 1 km long stream reaches in tributaries of the Yakima Basin during 2001 and 2002. Response variables in treatment reaches were compared to the same variables in control or reference sites (hereafter). Three types of reference sites were available for comparison, 1) adjacent upstream sites without analogs, 2) control streams where no analogs were stocked, and 3) identical sites that were measured in previous years (baseline data). Sampling occurred during four critical periods 1) before analogs were stocked, 2) 2 weeks after analogs were stocked (anticipated to be 50% carcass analog degradation), 3) 4 weeks after analogs were stocked (anticipated to be 90% carcass analog degradation, and the last reliable time to be able to collect fish in high elevation east-side streams), and 4) 1 year after analogs were stocked. Stable isotopes, stomach contents, fish size, prey, periphyton, water quality, and predators were sampled during 2001 stocking. Stomach contents, and pathogen samples were collected after the 2002 stocking. The biomass, abundance, growth, and condition of salmonids were measured before the analogs were introduced and 1 year after analog addition. Additional sampling during the winter and spring was not conducted because of limited access (snow) and the difficulty in capturing fish in cold or high water.

All of the stocking occurred in areas with few or no naturally occurring salmon carcasses to control for differential spawning escapement in different streams and stream sections. This meant that most of the study streams were in areas above partial or complete human made barriers and that the primary salmonid of interest was resident trout. Resident trout are a good representative of potential salmonid production because they do not move as much as salmon, are more abundant, and they eat similar prey as juvenile salmon (James et al. 1999). We assume that increased trout growth and survival will indicate the potential for increased salmon production in areas that are suitable for salmon migration and rearing.

Study streams

Six tributaries of the upper Yakima River were used for this study. Four of these tributaries (Wilson, Pearson, Coleman, and Cooke) drain the Colockum Mountain range and enter the Yakima River near the town of Ellensburg (Figure 1, Table 1). Historically these tributaries probably contained spawning steelhead *Oncorhynchus mykiss* and coho salmon, but presently support only resident fishes. The other two streams (West Fork and Middle Fork Teanaway River) flow into the Teanaway River from the northwest and the Teanaway River enters the Yakima River near Cle Elum. These two streams have small numbers of steelhead and Chinook salmon but probably had large numbers of contained steelhead, coho, and Chinook salmon *Oncorhynchus tshawytscha*. Analogs were used to mimic benefits provided by the coho and chinook salmon that historically spawned in these streams. Steelhead also are found in these

streams, but most migrate downstream after spawning and thus do not contribute carcass material at the study sites, but they do contribute eggs that may be eaten as well as emergent fry. Fish assemblages in these tributary streams are presently dominated by trout (e.g., rainbow trout *Oncorhynchus mykiss*, cutthroat trout *Oncorhynchus clarki*, brook trout *Salvelinus frontinalis*) and sculpins *(Cottus spp.)*.

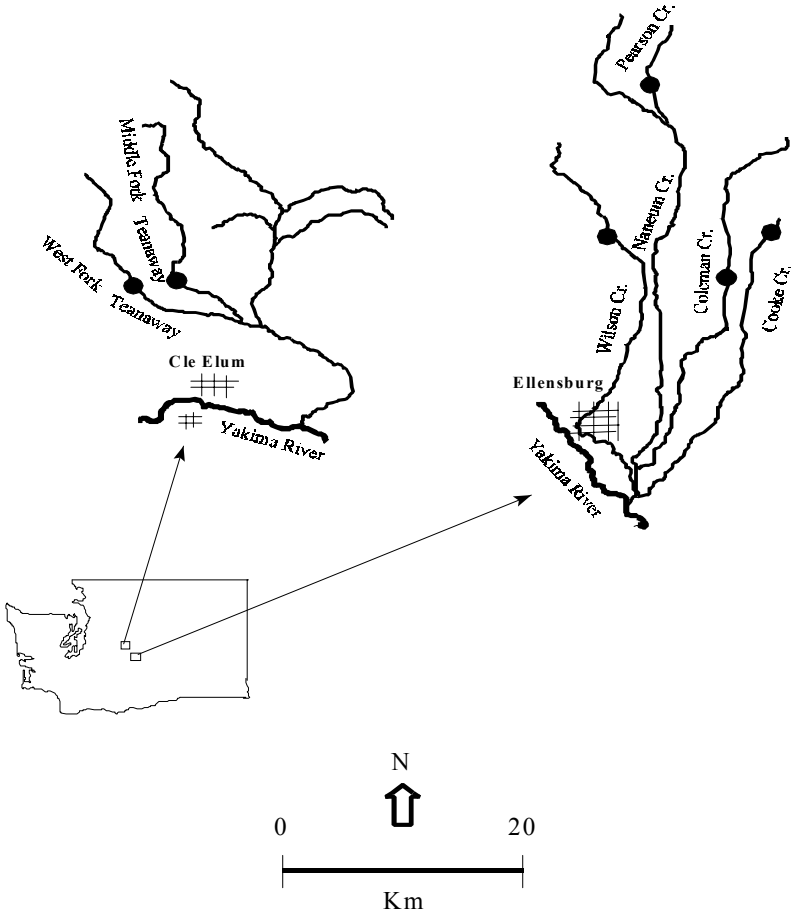

Figure 1. Locations of the six study streams.

Table 1. Locations of the middle sites in the six study streams.

Stream	Section	River Km.	Latitude	Longitude
Cooke	MPD[a]	26.21	47°15'46.7"N	120°57'1.1"W
Cooke	MPU[b]	27.21	47°15'50"N	120°57'39.29"W
Coleman	MPD[a]	21.38	47°16'44.1"N	120°55'47.8"W
Coleman	MPU[b]	22.38	47°16'52.9"N	120°56'26.8"W
Pearson	MPD[a]	2.25	47°14'59.9"N	120°27'23.1"W
Pearson	MPU[b]	3.35	47°15'25"N	120°27'45.5"W
Wilson	MPD[a]	34.68	47°9'52.8"N	120°30'37.1"W
Wilson	MPU[b]	35.68	47°10'18.2"N	120°30'43.1"W
MFT	MPD[a]	4.5	47°16'44.1"N	120°55'47.8"W
MFT	MPU[b]	5.5	47°16'52.9"N	120°56'26.8"W
WFT	MPD[a]	5.11	47°15'46.7"N	120°57'1.1"W
WFT	MPU[b]	6.11	47°15'50"N	120°57'39.29"W

[a] Middle Population Estimate site downstream.
[b] Middle Population Estimate site upstream.

Distribution of carcass analogs

Salmon carcass analogs were stocked into four tributaries in the Yakima Basin. Analogs were about the size of a large marshmallow and averaged 11.89g. Stocking occurred during September 19 and 20th, 2001 and September 18 and 19th, 2002 to correspond with natural spawn timing of spring chinook salmon. Four tributaries were treatment streams (Cooke, Coleman, Pearson, and West Fork Teanaway) and two were controls (Wilson Creek and the Middle Fork Teanaway River). Treatments consisted of stocking carcass analogs in a 1-km long stream section of each treated tributary. Analogs were stocked at densities of 30 g carcass analog material/m^2 of bank full channel width (Table 2). Stocking densities were derived from published relationships between salmon carcass densities and maximum stable isotope compositions. The amount of nutrients provided by carcasses was then adjusted for water weight. Analogs were placed into large buckets and evenly distributed throughout the reach. A reference section was established on each treated tributary upstream from the treated section. Control streams did not have analogs stocked into them. This design provided three references against which to compare the responses at the treated sites (e.g., upstream reference, control stream, and baseline references). Fish population data were previously collected at many of the study sites, providing important baseline information.

Table 2. Bankfull stream width, number of analogs stocked per lineal stream distance and the total weight of analogs stocked into each 1 km reach.

Stream	Bankfull Width(m)	Analog/m	Total kg
Coleman	5.26	13.27	765.6
Cooke	5.8	14.64	844.8
Pearson	4.93	23.45	717.2
WFT	15.74	39.7	2288
		Total	4615.6

Nitrogen and Carbon Stable Isotope Analysis

Carbon (C) and nitrogen (N) stable isotope ratios of fish muscle tissue were measured to determine the extent to which carcass analogs contributed to the diet of the fish at the treated sites. Additional stable isotope measurements to identify the important trophic pathways by which the analog material was incorporated into the stream ecosystem were at some of the sites. Macroinvertebrate and periphyton samples were analyzed at these locations. Stable isotope values of these samples enabled us to determine the extent to which these plants or animals are utilizing C and N from the analogs. These measures, in combination with the stable isotope values from the fish tissue samples, provide a clear indication of the overall influence of the analogs on the trophic dynamics of the treated stream reaches.

The relative contribution spawning salmon make to the nutrient pool in freshwater ecosystems can be quantified using N and C stable isotope analysis (Kline 1990; 1994; Bilby et al. 1996; 1998; Johnston et al. 1997). Spawning salmon (and the carcass analogs) contain higher proportions of the heavier isotopic form of both N and C (^{13}C and ^{15}N) than do N and C delivered to the stream from other sources. As a result, stable isotope ratios of these elements indicate the proportion of N and C of marine origin in a sample collected from the stream.

Samples were collected from each reference stream and from the treated reach and the control reach of each stream treated with carcass analogs shortly before adding the carcass analogs and approximately 4 weeks after. As C will not be added with the inorganic nutrients, C stable isotope analysis will not provide any meaningful information about the nutrient addition.

Fish, invertebrates and periphyton were collected for stable isotope analysis. Juvenile salmonids were collected for analysis in "collection sites" at all sites (Tables 3-5). Approximately 4 fish of the 2 most abundant salmonid species were collected at each site before analog stocking, 4 weeks after, and 1 year after stocking for stable isotope analysis. Samples for these analyses were not collected from population estimate sites. The target number of fish sampled was 288 (6 tributaries x 2 sites/tributary x 3 periods x 8 fish). The guts of the fish were evacuated by stomach lavage prior to euthanizing. Larger numbers of fish were not sampled to avoid any possible impacts on the population parameters also being measured.

We restricted our invertebrate analysis to grazing invertebrates to keep costs low and to follow the feeding pathway through the periphyton. Invertebrates were collected with a D-frame kick net and were held alive for 24 hours to allow for evacuation of gut contents. The invertebrates were then segregated into feeding types and frozen. The total number of

invertebrate samples we attempted to collect was 36 (6 tributaries x 2 sites/tributary x 3 sample periods).

The periphyton samples were scraped from streambed rocks using a razor blade and stiff-bristle brush. The material was rinsed from the rock into a plastic pan with a small volume of water. The periphyton was separated from the liquid by filtering thorough a pre-ashed, glass fiber filter. The total number of periphyton samples we attempted to collect was 36 (6 tributaries x 2 sites/tributary x 3 sample periods).

Fish, invertebrate, periphyton, and 10 analog samples were frozen. Frozen samples were shipped to the NMFS Northwest Fisheries Science Center for processing and subsequently sent to the University of Alaska for isotope sampling.

Preparation of fish samples for stable isotope analysis involved thawing, removal of approximately 10 g of dorsal muscle tissue, rinsing with distilled water and freeze drying. Invertebrate samples included the entire animal. Invertebrate and periphyton samples were also freeze dried. Dried samples were ground to a fine powder using a ball grinder and mortar and pestle. A small amount of the ground tissue (1-1.5 mg) was combusted. The evolved N_2 or CO_2 gas was introduced into a continuous flow isotope ratio mass spectrometer to determine $\delta^{13}C$ and $\delta^{15}N$ values. These values represent the difference in parts per thousand (‰) between the level of ^{13}C or ^{15}N in the sample and that in the standard. δ values were calculated:

$$\delta^{15}N/^{13}C = [(R_{sample}-R_{standard})/R_{standard}]\times 1000$$

where R_{sample} = the stable isotope ratio in the sample and $R_{standard}$ = the ratio in the standard. Standard for N is atmospheric N and for C is Peedee Belemnite, a calcareous rock from a formation in South Carolina, (Peterson and Fry 1987).

Periphyton biomass

Ceramic tiles were distributed in treated and untreated areas to determine periphyton biomass accumulation. Approximately 12 square tiles (229.84 cm^2) were placed within each of the lower 100 m of the upstream and downstream 1 km long reaches of each stream. Tiles were distributed on August 13, 2001 and retrieved on November 1 and 2, 2001. Periphyton was scraped from the tiles in the field, placed on ice, and then frozen (Table 4). AFDM was estimated using methods described by Steinman and Lamberti (1996).

Diet Analysis

Stomach contents were obtained from fish longer than 60 mm using gastric lavage. Samples were taken during the 4 sample periods. These sample times reflected the two different pathways that fish food is increased, 1) direct consumption of the analog and 2) increased consumption of invertebrates. After stomach contents were collected, the fish were retained for stable isotope analysis or released back into the stream. Approximately 30 fish were examined for each treatment or control section in each stream. Invertebrates in the guts were identified to order and carcass analog material was identified and separated (Tables 3-6). Invertebrates and

analog material were dried separately for 48 hours in a drying oven and weighed. Stomach contents were expressed as % full based on methods described by James et al. (1999).

Predators

Anecdotal comparisons of salmonid predators between treated and untreated sites were made to index instead whether the analogs promote an increase in these animals and if this increase had any appreciable impact on the fish. Aquatic and terrestrial predators were incidentally recorded during surveys described for the other tasks.

Pathogens

As possible disease transmission is a major concern related to carcass placement, we visually examined fish in the study reaches for the appearance of any external disease or parasite. In addition, fish samples that were collected for stable isotopes were analyzed for pathogens using the protocols identified by the wild fish survey, by the Lower Columbia Fish Health Center (Tables 3-6).

Water quality

Water quality impacts also have been raised as a concern relating to carcass placement. We took biweekly water samples at the downstream end of the reaches with and without analogs and from the reference tributary sites between August and October, 2001. Three samples were taken before and after analog addition. The total number of water samples was 72 (6 tributaries x 2 sites/tributary x 6 periods) (Tables 3-4). Measured water quality parameters were: dissolved oxygen, NO_3/NO_2-N; NH_4-N, and total phosphorus. Water chemistry samples were frozen and sent within one month of collection to the University of Washington for processing.

Fish assemblage composition, abundance, and biomass

Estimates of fish assemblage composition and biomass, and trout density were collected in 3 x 100 m long sample sites in upstream and downstream sites at each treated and untreated tributary (e.g., 6 sites/tributary). Estimates were conducted before and 1 year after analog addition (Tables 3 and 5). The 100 m long sample sites were located within the middle 800 m of the 1 km long study reach (Figure 2). Eliminating areas near the boundaries of the treated reaches as possible sample sites minimized potential confounding effects due to movement of fish between treated and untreated areas (buffers, Figure 2). Sites were located in the middle (historic site with extensive baseline data), and 100 m from the top and bottom ends. The specific 100-m sections to be sampled were established on the first sample date and the same sections were resampled throughout the study. Fish were sampled using backpack electrofishing. We attempted to net every fish encountered and identified to species and age class. Lengths and weights were

measured for every salmonid. Other fishes were counted, identified to species and age class and weighed in species-life-stage groups. Block nets were installed at the top and bottom ends of each 100 m site. A population estimate was generated using multiple removal methods before the analogs were stocked and 1 year after stocking (not presented in this report). Multiple electrofishing passes occurred until subsequent passes yielded less than 50% of the target fish larger than 79 mm during the previous pass. Population estimates were not conducted during other periods because of the unlikelihood that reliable estimates could be calculated at low water temperatures. Population estimates were calculated with the MicroFish program.

Buffer	Lower Pop	Lower Collection Site	Middle Pop	Upper Collection Site	Upper Pop	Buffer
100 M	100 M	250 M	100 M	250 M	100 M	100 M

Figure 2. Locations of buffers, population estimation reaches (Pop), and collection reaches within 1 km long treatment and control reaches (M=meters).

Size structure, growth and condition of salmonids

Approximately 75 age 1+-2+ rainbow trout were PIT tagged in the treatment and control reaches of MFT, WFT, Cooke, and Coleman Creeks about 1 month prior to analog addition (e.g., total of 300) (Table 2). At the end of October and during the following summer, we attempted to recapture these fish to determine growth of individual fish (Tables 4-6). Instantaneous growth was determined for all re-captured PIT tagged fish. The instantaneous growth rate for length and weight was calculated using the following equation (Ricker 1975):

$$IGR = (\log_e Y_2 - \log_e Y_1)/(t_2 - t_1)$$

where Y_2 = the weight (g) or fork length (mm)at the end of the period, Y_1 = the weight (g) or fork length at the beginning of the period, and $t_2 - t_1$ = the number of days at large.

Lengths and weights were measured for every salmonid sampled. Growth of non-PITtagged trout was evaluated by comparing the mean increase of fish size for each age class between sampling periods. Sampling occurred two weeks, four weeks, and one year after analog addition. Immediately prior to analog distribution a survey was conducted to provide a temporal reference. Two and four weeks after analog stocking, fish were collected in sites other than the population estimate sites and growth was determined. The proposed study design allowed us to compare control and treatment sections within a stream and control and treatment streams before and after analog distribution.

Movement and population turnover

Trout greater than 100 mm were marked with a fin punch in population estimate reaches to determine population turnover. A small fin punch was made with a small sized paper punch. The punch was made adjacent to the hypural plate on the lower caudal fin. In addition, movement of PIT tagged fish was assessed.

Degradation, invertebrate colonization, and rate of retention of analogs

Depending on access to sampling sites, analogs were examined periodically to determine the rate of decomposition, invertebrate colonization, and rate of retention within the stream channel. We attempted to weigh individual analogs, but this method proved to be unfeasible because the analog absorbed water weight (increased weight) and they broke apart when picked up. Temperature loggers were placed in treatment and control reaches to monitor temperature.

Table 3. Sample sizes of various parameters collected before analog placement 2001. Sec. =Section, D =Downstream, U =Upstream, Pop.Est. Population Estimate, Path. =Pathogen, Perip. = Periphyton, Chem. =Chemistry.

Stream	Sec.	Pop Est.	Fish Pit Tagged	# of Stomachs	Stomach invert I.D.[a]	Fish for Path.[b]	Stable Isotope fish[c]	Periph.	Water Chem.	Stable Isotope invert[d]
Cooke	D	3	69	15	7	8	8	1	3	1
Cooke	U	3	75	8	8	8	8	1	3	1
Coleman	D	3	77	8	8	8	8	1	3	1
Coleman	U	3	73	0	0	8	8	1	3	1
Pearson	D	3	0	7	7	8	8	1	3	1
Pearson	U	3	0	0	0	8	8	1	3	1
Wilson	D	3	0	8	8	8	8	1	3	1
Wilson	U	3	0	8	8	8	8	1	3	1
MFT	D	3	74	16	11	8	8	1	3	1
MFT	U	3	83	8	8	8	8	1	3	1
WFT	D	3	68	8	8	8	8	1	3	1
WFT	U	3	74	16	16	8	8	1	3	1

[a] The number of stomach samples keyed out to invertebrate order, [b] The number of fish collected for pathogen work, [c] The number of fish collected for stable isotope work, [d] The number of invertebrate samples collected for stable isotope work.

Table 4. Sample sizes of various parameters collected after analog placement 2001. Sec. =Section, D =Downstream, U =Upstream, Stom. =Stomachs, Periph. =Periphyton, Chem. =Chemistry.

Stream	Sec	Pit Tag Recaps	# of Stom.	Invert I.D.[a]	Fish for Path.[b]	Stable Isotope fish[c]	Periph.	Solar Path.[e]	Ash free dry mass[f]	Water Chem.	Stable Isotope invert[d]
Cooke	D	21	115	30	8	8	1	10	10	3	1
Cooke	U	32	58	25	8	8	1	8	8	3	1
Coleman	D	17	113	42	8	8	1	7	11	3	1
Coleman	U	25	58	35	8	8	1	4	12	3	1
Pearson	D	0	108	47	8	8	1	9	11	3	1
Pearson	U	0	46	8	8	8	1	6	11	3	1
Wilson	D	0	52	37	8	8	1	12	12	3	1
Wilson	U	0	46	38	8	8	1	11	12	3	1
MFT	D	13	62	21	8	9	1	6	6	3	1
MFT	U	24	42	23	8	8	1	8	8	3	1
WFT	D	13	246	111	8	12	1	11	11	3	1
WFT	U	16	81	18	8	12	1	12	12	3	1

[a] The # of stomach samples keyed out to invertebrate order, [b] The number of fish collected for pathogen work, [c] The number of fish collected for stable isotope work, [d] The number of invertebrate samples collected for stable isotope work, [e] The number of solar pathfinder readings taken. [f] The number of tile ash free dry mass samples.

Table 5. Sample sizes of various parameters collected before analog placement 2002. Sec. =Section, D =Downstream, U =Upstream, Pop. Est. =Population Estimate, Periph. =Periphyton.

Stream	Sec.	Pop. Est.	Pit Tag Recaps	# of Stomachs	Invert I.D.[a]	Fish for Pathogen[b]	Stable Isotope fish[c]	Periph.	Solar Path finder	Stable Isotope invert[d]
Cooke	D	3	5	32	30	8	8	1	11	1
Cooke	U	3	6	64	30	8	8	1	10	1
Coleman	D	3	13	48	30	8	8	1	10	1
Coleman	U	3	4	22	30	8	8	1	11	1
Pearson	D	3	0	45	30	8	8	1	11	1
Pearson	U	3	0	48	30	8	8	1	12	1
Wilson	D	3	0	52	30	8	8	1	10	1
Wilson	U	3	0	36	30	8	8	1	10	1
MFT	D	3	0	39	31	8	8	1	11	1
MFT	U	3	1	35	30	8	8	1	10	1
WFT	D	3	1	42	32	8	8	1	10	1
WFT	U	3	2	26	30	8	8	1	10	1

[a] The number of stomach samples keyed out to invertebrate order, [b] The number of fish collected for pathogen work, [c] The number of fish collected for stable isotope work, [d] The number of invertebrate samples collected for stable isotope work.

Table 6. Sample sizes of various parameters collected after analog placement 2002. D =Downstream, U =Upstream.

Stream	Section	Pit Tagged Fish	# of Pit Tag Recaps	# of Stomachs	Invert I.D.[a]	Fish for Pathogen
Cooke	D	91	43	112	30	8
Cooke	U	56	50	142	30	8
Coleman	D	90	63	186	30	8
Coleman	U	130	80	232	30	8
Pearson	D	0	0	90	30	8
Pearson	U	0	0	99	30	8
Wilson	D	0	0	145	60	8
Wilson	U	0	0	132	65	8
MFT	D	0	2	111	31	8
MFT	U	0	2	115	31	8
WFT	D	0	0	123	30	8
WFT	U	0	1	121	30	8

[a] The number of stomach samples keyed out to invertebrate order.

Results

Degradation and performance of the analog

Overall, the behavior of the analog was excellent. The analogs were easy to transport and distribute throughout the stream channel. The analogs generally sank to the bottom and were retained within the channel. The size of the analogs facilitated the retention within the channel because they were small enough to be trapped by roughness elements (e.g., rocks) but large enough not to sink into interstices of rocks making them unavailable to species that live above the substrate. In addition, the analogs broke down at similar rates as spring chinook salmon carcasses in the upper Yakima Basin. Approximately 50% of the analog had dissolved or been eaten 2 weeks after stocking, and the analog was nearly gone after 4 weeks. Analogs were often colonized by what appeared to be a matrix of fungi and bacteria which produced a rubbery "skin" that was difficult to penetrate for about a week. Later on (approximately week 3) periphyton began to grow on the analogs and the analogs appeared as small piles of fine material. Few invertebrates were observed on the analogs. Temperatures were relatively warm in 2001, which probably increased the rate of degradation.

During 2002, some of the analogs floated because they had less moisture content than those stocked in 2001. Approximately, 15-20% of the bags in 2002 contained analogs that floated. In general, the analogs that floated traveled approximately 30 m before they were retained in the channel, and subsequently absorbed water, and sank. Some of the analogs may have traveled up to about 100 meters. Despite the lowered moisture content in 2002, we still observed significant condensation within the bags when we stored them in our un-refrigerated shop. We also observed that the analogs produced in 2001 molded in the bag if they were kept for over a month. The presence of the mold may have been due to moisture content that was too high or the method in which we stored the analogs.

Periphyton

Preliminary results indicate that periphyton biomass was higher in fertilized areas , presumably as a result of the analog addition. The AFDM (mg/cm^2) of periphyton on tiles was higher in the downstream sites of the treatment streams but not in the control streams (Table 7).

Table 7. Sample size and AFDM (mg/cm^2) of periphyton on tiles in treatment and control streams.

Stream	Upstream		Downstream	
	N	Mean	N	Mean
		Treatment Streams		
Coleman	12	0.44	11	0.54
Cooke	8	0.49	9	0.84
WFT	7	0.35	10	0.51
Pearson	11	0.31	10	0.65
Treatment Mean		0.40		0.63
		Control Streams		
MFT	6	0.15	5	0.12
Wilson	12	0.86	12	0.36
Control Mean		0.51		0.24

Stomach composition/diet

Rainbow/steelhead, cutthroat trout, and spring chinook salmon consumed the analog directly and often gorged themselves. The trout appeared to consume more of the analog than the salmon. This was very encouraging because it was this part of the food pathway that we were most uncertain about.

Trout, and to a lesser degree salmon, consumed the analog and increased their stomach fullness. The percent stomach fullness was similar in upstream and downstream sites in control streams and in sites before analog introduction (Table 8). After the analog was introduced the fullness was much higher in the treatment sites than in the control sites. The percent stomach fullness was generally less than 20% for all species before the analogs were introduced. The fullness was also less than 20% for all times and species, in sites and streams where analogs were not stocked. In contrast, the fullness of trout in treatment sites was over 20% within 2 weeks of stocking analogs. The % fullness of fish in the treatment sites decreased as the analog availability decreased (Table 8). The proportion of trout in treatment streams that contained the analog, within 2 weeks of stocking, ranged from 40 to 75% (Table 9) and 32% of the chinook salmon had analog in their gut during the same period.

Of the trout that ate the analog, the dry weight of the analog was heavier than the weight of the other stomach contents. However, the trout that ate the analog generally had more

invertebrate material than fish in control sites. This indicates that the analog did not replace other food types, but that it was eaten in addition to other food types. In addition, the analog may have stimulated more feeding on other prey types or at least made it more available to trout. Alternatively, the high gut fullness increased digestion time of invertebrates. Chinook salmon ate less of the analog than invertebrates (Table 10).

Stable Isotopes

Preliminary qualitative examination of the data suggests that mayflies were directly or indirectly consuming the analog. Stable isotope ratios for all of the sample periods are not available at this time. Unfortunately, we collected some of our periphyton samples in 2001 on coffee filters which made them unusable for analysis. All samples were collected on glass fiber filters in 2002.

Growth

The growth data are currently being analyzed.

Population estimates

Population estimates during the summer prior to analog addition are in (Tables 11-18). The population estimates for 2002 will be presented in a subsequent report.

Table 8. Average percent fullness of fishes sampled within control and treatment stream sections (upstream and downstream respectively). Sample periods include those before analog placement: 22 August-18 September 2001 (Before), one-two weeks after analog placement: 24 September-08 October 2001 (After 1st), and four weeks after analog placement: 22 October 2001 (After 2nd).

Species	Section	Before	N	After 1st	N	After 2nd	N
				Treatment Streams			
				Coleman			
Rainbow trout	Upstream	-	0	10.9	38	3.1	4
Rainbow trout	Downstream	21.1	4	29.9	60	7.9	4
				Cooke			
Rainbow trout	Upstream	6.6	8	6.2	46	4.8	8
Rainbow trout	Downstream	6.6	15	37.5	62	9.2	7
				WFT			
Rainbow trout	Upstream	14.2	16	10.6	55	14.1	8
Rainbow trout	Downstream	6.0	8	55.4	81	49.3	8
Chinook Salmon	Upstream	-	0	11.5	16	13.0	4
Chinook Salmon	Downstream	-	0	23.7	63	25.2	4
				Pearson			
Cutthroat trout	Upstream	-	0	8.0	37	7.0	8
Cutthroat trout	Downstream	15.5	7	46.4	64	15.9	9
				Control Streams			
				MFT			
Rainbow trout	Upstream	5.9	8	7.2	41	16.4	8
Rainbow trout	Downstream	8.7	16	7.8	45	6.6	7
Chinook Salmon	Upstream	-	0	19.0	2	-	0
Chinook Salmon	Downstream	-	0	14.8	12	19.5	2
				Wilson			
Cutthroat trout	Upstream	5.1	8	6.5	38	4.7	8
Cutthroat trout	Downstream	7.6	4	6.4	36	4.2	4

Table 9. Number of samples within each downstream/treatment section found to contain observable amounts of carcass analog. Sample periods include those one-two weeks after analog placement: 24 September-08 October (After 1st), and four weeks after analog placement: 22 October 2001 (After 2nd).

Species	After 1st			After 2nd		
	N	Number of samples with analog present	Percent of total sample with analog present.	N	Number of samples with analog present	Percent of total sample with analog present.
			Treatment Streams			
			Coleman			
Rainbow trout	60	24	40.0	4	0	0
			Cooke			
Rainbow trout	62	43	69.4	7	2	28.6
			WFT			
Rainbow trout	81	61	75.3	8	5	62.5
Chinook Salmon	63	20	31.7	4	0	0
			Pearson			
Cutthroat trout	64	39	60.9	9	4	44.4

Table 10. Breakdown of stomach fullness between carcass analog and invertebrate/other. Samples consist of all fishes, within treatment streams, found to contain observable amounts of carcass analog. Sample periods include those one-two weeks after analog placement: 24 September-08 October (After 1st), and four weeks after analog placement: 22 October 2001 (After 2nd).

Species	Sample period	Average percent fullness: Carcass analog	Average percent fullness: Other	Average percent fullness: Total	*N*
		Coleman			
Rainbow trout	After 1st	47.8	10.6	58.4	24
Rainbow trout	After 2nd	-	-	-	0
		Cooke			
Rainbow trout	After 1st	35.3	12.5	47.8	43
Rainbow trout	After 2nd	11.4	2.2	13.6	2
		WFT			
Rainbow trout	After 1st	51.4	15.4	66.8	61
Rainbow trout	After 2nd	42.1	18.0	60.1	5
Chinook salmon	After 1st	10.6	22.5	33.1	20
Chinook salmon	After 2nd				0
		Pearson			
Cutthroat trout	After 1st	57.5	12.1	69.6	39
Cutthroat trout	After 2nd	23.7	5.7	29.4	4

Table 11. Population estimates for treatment streams containing rainbow trout.

	Density			Biomass		Fork length
Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
Coleman			Treatment streams containing Rainbow trout			
LPU	46	40 - 58	0.625	731.4	15.9	106
MPU	29	29 - 31	0.853	661.0	22.8	120.6
UPU	58	52 - 69	0.667	1307.2	22.5	122
Mean:	44.3	8 - 80	0.715	899.9	20.4	116.2
S.D:	14.6		0.121	354.5	3.9	8.9
LPD	35	35 - 36	0.921	731.0	21	118.5
MPD	38	37 - 42	0.804	835.0	22	120
UPD	76	72 - 76	0.758	1817.7	23.9	122
Mean:	49.7	7 - 106	0.828	1127.9	22.3	120.2
S.D:	22.9		0.084	599.6	1.5	1.8
Cooke						
LPU	33	32 - 37	0.8	1113.8	33.8	137.8
MPU	67	62 - 76	0.713	2461.7	36.7	141.3
UPU	48	46 - 53	0.78	1640.3	34.2	137.8
Mean:	49.3	7 - 91	0.764	1738.6	34.9	139.0
S.D:	17.0		0.046	679.3	1.6	2.0
LPD	54	49 - 64	0.681	1834.9	34	138.2
MPD	70	67 - 76	0.779	1967.3	28.1	128
UPD	37	35 - 42	0.745	1167.1	31.5	132
Mean:	53.7	12 - 94	0.735	1656.4	31.2	132.7
S.D:	16.5		0.050	428.9	3.0	5.1
WFT						
LPU	29	29 - 31	0.879	943.0	32.5	139
MPU	26	24 - 33	0.686	642.4	24.7	127.7
UPU	48	38 - 68	0.535	1581.5	32.9	136.1
Mean:	34.3	4 - 64	0.700	1055.6	30.0	134.3
S.D:	11.9		0.172	479.6	4.6	5.9
LPD	21	18 - 30	0.45	546.0	26	130.8
MPD	66	58 - 79	0.644	2156.4	32.7	139.5
UPD	38	35 - 45	0.70	1240.0	32.6	137.4
Mean:	41.7	-15 - 98	0.598	1314.1	30.4	135.9
S.D:	22.7		0.131	807.8	3.8	4.5
			Means for treatment streams containing rainbow trout			
Mean (U)	42.7	31 - 53	0.726	1231.4	28.4	125.9
S.D:	14.3		0.112	594.6	7.1	12.4
Mean (D)	48.3	34 - 63	0.720	1366.2	28.0	125.6
S.D:	18.9		0.129	594.1	5.0	9.2

Table 12. Population estimates for treatment stream containing brook trout.

	Density			Biomass		Fork length
Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
Coleman			Treatment stream containing brook trout			
LPU	12	11 - 18	.647[a]	188.7	15.7	109.8
MPU	15	12 - 28	.522[a]	228.8	15.3	111.2
UPU	8	8 - 10	.866	217.0	27.1	119.6
Mean:	11.7	8-15	.678	211.5	19.4	113.5
S.D:	2.86		.142	16.8	5.47	4.3
LPD	14	13 - 19	.684	352.2	25.2	125.6
MPD	17	16 - 22	.696	422.9	24.9	121.0
UPD	15	14 - 20	.700	566.8	37.8	135.9
Mean:	15.3	14 - 17	.693	447.3	29.3	127.5
S.D:	1.52		.007	89.3	6.01	6.23

[a] These capture probabilities are too small to generate an accurate population estimate.

Table 13. Population estimates for treatment stream containing cutthroat trout.

	Density			Biomass		Fork length
Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
Pearson			Treatment stream containing cutthroat trout			
LPU	97	94 – 103	.810	1982.3	20.4	119.0
MPU	127	126 – 130	.887	2402.9	18.9	114.9
UPU	34	33 – 38	.805	493.5	14.5	108.6
Mean:	86	32 – 140	.834	1626.2	17.9	114.2
S.D:	47.5		.045	1003.2	3.06	5.2
LPD	82	78 – 89	.765	1460.2	17.8	112.4
MPD	89	89 – 91	.927	1745.0	19.6	116.3
UPD	82	81 – 85	.862	1915.4	23.4	120.9
Mean:	84.3	79 – 89	.851	1706.9	20.3	116.5
S.D:	4.04		.081	229.9	2.85	4.3

Table 14. Population estimates for treatment streams containing salmonids.

Site	Density (Fish/ 100m)	95 % CI	Capture probability	Biomass g / 100 m	Mean weight (g)	Fork length Mean FL (mm) Fish > 79mm
Combined treatment streams						
Downstream Reaches						
Mean:	48.9	35 – 63	0.741	1250.5	26.7	126.5
S.D:	27.3		0.101	649.8	6.03	8.0
Upstream Reaches						
Mean:	45.1	29 – 61	.762	1106.4	24.5	123.4
S.D:	32.3		.108	765.1	7.85	12.3

Table 15. Population estimates for control stream containing rainbow trout.

Site	Density (Fish/ 100m)	95 % CI	Capture probability	Biomass g / 100 m	Mean weight (g)	Fork length Mean FL (mm) Fish > 79mm
Middle Fork Teanaway	Control stream containing rainbow trout					
LPU	47	41 – 58	0.482	1444.4	30.7	130.0
MPU	65	64 – 68	0.853	1741.8	26.8	124.4
UPU	45	41 – 54	0.683	1004.3	22.3	121.6
Mean:	52.3	25 – 79	0.673	1396.8	26.6	125.3
S.D:	11.0		0.186	371.0	4.2	4.3
LPD	83	77 – 92	0.720	3046.2	36.7	141.0
MPD	77	69 – 89	0.670	1885.9	24.5	127.0
UPD	32	32 – 34	0.889	941	29.4	130.7
Mean:	64	5 – 133	0.760	1957.7	30.2	132.9
S.D:	27.9		0.115	1054.4	6.1	7.3

Table 16. Population estimates for control stream containing cutthroat trout.

Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
Wilson			Control stream containing cutthroat trout			
LPU	68	63 - 77	0.716	2128.5	31.3	132.0
MPU	53	53 - 55	0.898	2145	40.5	144.7
UPU	57	54 - 64	0.750	1286.7	22.6	118.7
Mean:	59.3	40 - 78	0.788	1853.4	31.5	131.8
S.D:	7.8		0.097	490.8	9.0	13.0
LPD	75	72 - 81	0.783	1477.1	19.7	116.7
MPD	52	50 – 57	0.781	980.7	18.9	115.4
UPD	85	79 – 94	0.725	1543	18.2	115.0
Mean:	70.7	28 – 112	0.763	1333.6	18.9	115.7
S.D:	16.9		0.033	307.4	0.8	0.9

Table 17. Population estimates for control stream containing brook trout.

Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
Wilson			Control stream containing brook trout[a]			
LPD	32	27 – 45	.587	656.6	20.5	120.4
MPD	16	16 – 19	.800	264.0	16.5	106.3
UPD	44	40 – 53	.678	1210.0	27.5	126.2
Mean:	31	15 – 47	.688	710.2	21.5	117.6
S.D:	14.05		.11	475.3	5.57	10.2

[a] There were no Brook trout in any of the upstream sites.

Table 18. Population estimates for all salmonids in the control streams.

Site	(Fish/ 100m)	95 % CI	Capture probability	g / 100 m	Mean weight (g)	Mean FL (mm) Fish > 79mm
			Combined Upstream and Downstream Sites			
Upstream						
Mean	55.83	48 – 72	.730	1625.1	29.03	128.6
SD	9.35		.147	462.6	6.8	9.4
Downstream						
Mean	55.1	38 – 63	.737	1333.8	23.5	122.1
SD	25.7		.088	806.1	6.6	10.3
			All Control Stream Sites Combined			
Control Streams Mean	55.4	45 – 63	.734	1450.3	25.74	124.7
SD	20.2		.110	685.3	7.01	10.1

[a] Control stream reaches are designated upstream or downstream. In treatment streams these are equivalent to control and treatment reaches respectively.

Pathogens

There was no difference in the incidence of pathogens that could be attributed to the analog addition. All of the fish tested negative for the following pathogens: (IPNV Infectious Pancreatic Necrosis Virus, IHNV Infectious Hematopoietic Necrosis Virus, VHS Viral Hemorrhagic Septicemia Virus, AS Furunculosis (*Aeromonas salmonicida*), YR Enteric Redmouth (*Yersinia ruckeri*), BCD Coldwater Disease (*Flexibacter psychrophilum*), CD Columnaris (*Flexibacter columarius*), ESC Emphysematous Putrefactive Disease (*Edwardsiella ictaluri)*, WD Whirling Disease (*Myxobolus cerebralis*), CS Salmonid Ceratomyxosis (*Ceratomyxa shasta*). *Renibacterium salmoninarum* was the only pathogen that was regularly found (Table 19). However, no difference in the occurrence of *R. salmoninarum* was observed in before/after samples or between treatment and control sites. *Henneguya* spores were found in the heads of some of the fish. Our visual examination of the fish also support the finding that the fish were healthy.

Table 19. Percentage of fish testing positive for RS BKD before and after carcass analog placement 2001.

Stream	Site	Time	*N*	% RS
		Treatment		
Coleman	Upstream	Before	8	0
Coleman	Upstream	After	8	0
Coleman	Downstream	Before	8	0
Coleman	Downstream	After	8	0
Cooke	Upstream	Before	4	12.5
Cooke	Upstream	After	4	0
Cooke	Downstream	Before	4	0
Cooke	Downstream	After	4	12.5
Pearson	Upstream	Before	8	0
Pearson	Upstream	After	8	0
Pearson	Downstream	Before	8	12.5
Pearson	Downstream	After	8	0
WFT	Upstream	Before	8	12.5
WFT	Upstream	After	8	0
WFT	Downstream	Before	8	12.5
WFT	Downstream	After	8	12.5
		Control		
MFT	Upstream	Before	8	25
MFT	Upstream	After	8	0
MFT	Downstream	Before	8	0
MFT	Downstream	After	8	0
Wilson	Upstream	Before	8	25
Wilson	Upstream	After	8	12.5
Wilson	Downstream	Before	4	0
Wilson	Downstream	After	4	0

Movement/turnover

Movement of fish during the summer and fall appeared to be small. Most of the fish that were PIT tagged and recaptured were caught within the reach that they were tagged (Table 20).

Table 20. Rainbow trout PIT tagged during 2001 field season. PIT tagging occurred between 8/1 and 8/9 2001. Fish were re-captured from 10/16 and 10/18 2001.

	Site of PIT tagging				
	LCD	UCD	LCU	UCU	*TOTALS*
			Coleman		
Number of fish PIT tagged	36	41	44	29	150
Number of fish re-captured (within site [a] / outside site)	14 (38%) / 0 (0%)	3 (7.3%) / 1 (2.4%)	14 (31.8%) / 0 (0%)	11 (38%)/ 0 (0%)	42 (28%) / 1 (.6%)
Distance moved (range in meters)	0-200	113-363 [b]	0-200	0-200	10-215
Seconds shocked on re-cap run	2917	1060	2402	2530	8909
Meters shocked on re-cap run	225	150	250	250	875
			Cooke		
Number of fish PIT tagged	37	32	39	36	144
Number of fish re-captured (within site [a] / outside site)	6 (16.2) / 2 (5.4%)	15 (47%) / 2 (6.3%)	21 (54%) / 0 (0%)	11 (30.5%) / 0 (0%)	53 (37%) / 4 (2.7%)
Distance moved (range in meters)	38-288 [b]	18-241 [b]	0-200	0-200	14-298
Seconds shocked on re-cap run	2996	1630	3033	1876	9535
Meters shocked on re-cap run	250	150	250	250	900
			Middle Fork Teanaway		
Number of fish PIT tagged	50	24	43	40	157
Number of fish re-captured (within site [a] / outside site)	10 (20%) / 2 (.4%)	3 (12.5%) / 1 (4.1%)	19 (44%) / 0 (0%)	5 (12.5%) / 0 (0%)	37 (23.5%) / 3 (1.9%)
Distance moved (range in meters)	25-258 [b]	38-288 [b]	0-200	0-200	10-210
Seconds shocked on re-cap run	3379	984	2883	1260	8506
Meters shocked on re-cap run	250	150	250	100	750
			West Fork Teanaway		
Number of fish PIT tagged	68		74		142
Number of fish re-captured (within site [a] / outside site)	13 (19%) / 6 (8.8%)		16 (21%) / 2 (2.7%)		29 (20.4%) / 8 (5.6%)
Distance moved (range in meters)	32-266 [b]		750-267 [b]		31-229
Seconds shocked on re-cap run	2607	1826	2109		6542
Meters shocked on re-cap run	250	250	250		750

[a] *Site where the fish was PIT tagged*

[b] *Average for fish that remained in the site and those that moved*

L,U = Lower and Upper sites respectively

C = Collection sites

C,T = Control and Treatment reaches respectively

*(e.g. LCT = **L**ower **C**ollection site of the **T**reatment reach).*

Water Quality

The water quality of control sites and treatment sites are presented in (Table 21). Preliminary interpretations of the water quality data suggested that the nutrients provided by the analogs were quickly taken up within the stream reach and little if any was transported out of the reach. However, there was a film of residue that was temporarily observed on the surface of the slow water which may have been caused by the fat in the analogs.

TABLE 21. Chemical analysis of water samples collected at each upstream (U) and downstream (D) location before and after analog placement. Downstream sections of treatment streams were stocked with carcass analog on September 19th and 20th 2001. Dissolved nutrient concentrations expressed in mg/L; **TP** Total phosphorous, **TN** Total nitrogen, **NO3-N** Nitrate-nitrogen, **NO2-N** Nitrite-nitrogen, **NH4-N** Ammonium-nitrogen concentrations. Samples for TP and TN were unfiltered, and the others were filtered.

Sample	TP		TN		NO3-N		NO2-N		NH4-N	
Site/Date	D	U	D	U	D	U	D	U	D	U
Coleman					Treatment					
8/13	0.0710	0.0706	0.136	0.159	0.010	0.011	0.0006	0.0005	0.0047	0.0036
8/27	0.0642	0.0642	0.132	0.162	0.038	0.036	0.0006	0.0006	0.0019	0.0017
9/10	0.0728	0.0640	0.478	0.210	0.033	0.032	0.0006	0.0006	0.0030	0.0027
9/24	0.0614	0.0602	0.142	0.101	0.013	0.007	0.0004	0.0004	0.0142	0.0041
10/8	0.0540	0.0521	0.084	0.071	0.001	0.000	0.0001	0.0001	0.0035	0.0005
10/22	0.0491	0.0540	0.082	0.100	0.000	0.000	0.0002	0.0002	0.0033	0.0031
Cooke					Treatment					
8/13	0.0706	0.0730	0.153	0.152	0.057	0.038	0.0006	0.0006	0.0023	0.0032
8/27	0.0767	0.0641	0.481	0.169	0.068	0.069	0.0010	0.0006	0.0019	0.0020
9/10	0.0633	0.0618	0.159	0.156	0.061	0.064	0.0004	0.0004	0.0007	0.0020
9/24	0.0660	0.0574	0.188	0.113	0.048	0.033	0.0007	0.0005	0.0209	0.0037
10/8	0.0541	0.0685	0.081	0.150	0.000	0.000	0.0001	0.0001	0.0035	0.0008
10/22	0.2509	0.0588	-0.110	0.130	0.002	0.000	0.0003	0.0002	0.0037	0.0024
Pearson					Treatment					
8/13	0.0389	0.0325	0.125	0.102	0.011	0.028	0.0003	0.0003	0.0039	0.0032
8/27	0.0297	0.0238	0.142	0.069	0.015	0.013	0.0003	0.0003	0.0015	0.0012
9/10	0.0279	0.0279	0.080	0.087	0.011	0.007	0.0003	0.0003	0.0025	0.0013
9/24	0.0335	0.0279	0.122	0.110	0.022	0.009	0.0004	0.0003	0.0159	0.0035
10/8	0.0245	0.0240	0.063	0.051	0.002	0.000	0.0001	0.0001	0.0023	0.0001
10/22	0.0281	0.0283	0.094	0.081	0.000	0.000	0.0002	0.0002	0.0052	0.0025
West Fork Teanaway					Treatment					
8/13	0.0125	0.0119	0.047	0.054	0.001	0.000	0.0001	0.0000	0.0008	0.0031
8/27	0.0133	0.0137	0.064	0.064	0.000	0.000	0.0003	0.0002	0.0005	0.0000
9/10	0.0136	0.0140	0.065	0.071	0.000	0.000	0.0003	0.0003	0.0013	0.0026
9/24	0.0109	0.0292	0.046	0.247	0.001	0.028	0.0002	0.0002	0.0023	0.0330
10/8	0.0108	0.0094	0.049	0.032	0.003	0.000	0.0001	0.0001	0.0050	0.0002
10/22	0.0146	0.0162	0.065	0.104	0.003	0.004	0.0002	0.0002	0.0016	0.0029
Middle Fork Teanaway					Control					
8/13	0.0123	0.0114	0.070	0.052	0.004	0.000	0.0001	0.0006	0.0023	0.0009
8/27	0.0119	0.0106	0.099	0.056	0.000	0.000	0.0002	0.0004	0.0001	0.0006
9/10	0.0118	0.0117	0.056	0.055	0.000	0.000	0.0002	0.0002	0.0013	0.0018
9/24	0.0141	0.0100	0.078	0.042	0.003	0.000	0.0002	0.0002	0.0023	0.0038
10/8	0.0086	0.0092	0.032	0.035	0.000	0.000	0.0001	0.0001	0.0004	0.0004
10/22	0.0121	0.0136	0.059	0.097	0.000	0.000	0.0002	0.0002	0.0022	0.0024
Wilson					Control					
8/13	0.0542	0.0535	0.109	0.099	0.019	0.019	0.0003	0.0003	0.0028	0.0023
8/27	0.0506	0.0493	0.133	0.113	0.021	0.022	0.0003	0.0003	0.0006	0.0006
9/10	0.0485	0.0493	0.086	0.088	0.017	0.015	0.0004	0.0004	0.0065	0.0020
9/24	0.0522	0.1104	0.093	0.300	0.009	0.010	0.0002	0.0002	0.0015	0.0029
10/8	0.0472	0.0441	0.066	0.051	0.001	0.003	0.0001	0.0001	0.0008	0.0009
10/22	0.0548	0.0491	0.102	0.081	0.000	0.000	0.0002	0.0002	0.0016	0.0025

Predators

Very few terrestrial predators were observed during the course of the study and the abundance of aquatic predators did not change. However, bear scat was observed in the West Fork Teanaway site.

Discussion

Our preliminary results suggest that the introduction of carcass analogs into potentially food limited streams can be used to restore food pathways previously provided by anadromous salmon. The analogs probably reproduced both of the major food pathways that salmon carcasses produce: direct consumption and food chain enhancement. Trout and salmon both fed directly on the carcass analogs during the late summer and presumably benefited from the increased invertebrate biomass later in the year. Future reports will analyze whether any benefits are statistically detectable. The risks of using carcass analogs also appear to be low. Pathogens appear to be killed in the manufacturing process of the analogs. In addition, preliminary results suggest that fish exposed to the analogs did not have higher incidences of pathogens. The water quality was also not degraded by the analog additions with the exception of a temporary surface film. Finally, our anecdotal observations, suggested that there was not an increase in the number of predators during the first year of analog distribution. In summary, the risks of analog placement appear to be low but the potential benefits appear to be high.

Rainbow trout appear to directly consume the analog more than spring chinook salmon. This same pattern of consumption has also been observed relative to natural spring chinook carcasses in the Yakima River (WDFW, unpublished data). This may be due to a difference in interspecies selectivity or because spring Chinook are generally smaller than most of the trout that we sampled.

Carcass analogs compare favorably to other methods of food enhancement. We think that the amount of work to distribute analogs was probably similar to that for distributing inorganic nutrients such as the “silver bullets” used in British Columbia and was considerably less work than stocking hatchery salmon carcasses. The analogs might simulate the natural food pathways better than inorganic nutrients because they provide a direct food source in the fall, similar to carcasses. Carcass analogs also present fewer pathogen risks than stocking salmon carcasses, are relatively easy to store, and are more readily available to stock into areas without salmon hatcheries or in areas where salmon hatchery carcasses are unable to meet the nutrient need. In summary, we believe that carcass analogs have the following desirable characteristics which, in combination, are not provided by any other nutrient addition technique: carcass analogs mimic natural food pathways, are easy to store and transport, may be available in large quantities, and pose low risk to aquatic communities.

The best way to restore historic food pathways is to restore salmon to their historic abundance and distribution. However, it is unlikely that we will ever return to historic abundances. Thus, we may need to embark on a periodic or continual nutrient addition program in order to restore marine derived nutrients. Distribution of carcass analogs appear to be a quasi-normative method of re-establishing important food pathways. Where escapement is not managed for nutrient needs (Bilby et al. 2001), carcass analogs might be used to provide needed nutrients.

Multiple years of stocking carcass analogs may be necessary before the full benefits to aquatic systems are observed. For instance, invertebrate populations that are small may need a few years to fully respond to the elevated food abundance in the stream.

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Appendix 1.

Objective 1 Bio-Oregon: Develop and produce a pathogen free salmon carcass analog from marine fish wastes.

Task1.1. Produce pathogen free salmon fishmeal.

Bio-Oregon produced salmon fishmeal in the fall of 1999, 2000, and 2001 from fall chinook salmon (*Oncorhynchus tschawytcha*) carcasses from Spring Creek National Fish Hatchery (NFH), Underwood, Washington, to meet the needs of this study. The salmon fishmeal was made in the following manner. The fresh, raw carcasses were coarsely ground and dried to a meal using swept surface, steam-tube dryers. In 1999 and 2000, each dryer (batch) was loaded with about 2,400 pounds of raw, ground salmon. In 2001 we had many more carcasses to process daily so we needed to load the dryers with about 3,300 pounds of raw, ground salmon. Liquid ethoxyquin was added (0.015%) to each batch prior to drying to prevent lipid oxidation. The steam temperature was a minimum of 250 F (121 C). The drying process, with steam on, took an average of 7.8 hours. The mass of ground carcasses reached 185-200 F (85-93 C) in about two hours. This temperature persisted for about five hours or until the drying material reached a moisture of about 20%. At that time the temperature began to climb, approaching 212 F (100 C) as the moisture level neared 10%. The steam was then turned off, but the sweeping mechanism continued to mix the meal until it cooled to less than 90 F (32 C). This took about four hours. At that time the meal was removed from the dryer and placed in bulk bags for storage.

Bio-Oregon produced 7,400 pounds of salmon fishmeal from 38,181 pounds of fall chinook carcasses in the fall of 1999 and 2000, which equals a yield of 19.4%. In the fall of 2001, 29,599 pounds of fishmeal was produced from 129,922 pounds of fall chinook carcasses, which equals a yield of 22.8%. The greater yield in 2001 occurred because many of the adult fall chinook were not spawned, but were killed soon after entering Spring Creek NFH. Because of this, the fish contained more lipids and less moisture, which resulted in a greater yield when the carcasses were dried to a meal.

The cooking/drying conditions (times/temperatures) described above easily exceed those of the standard pasteurization conditions that have been employed by Bio-Oregon for the last 40 years to pasteurize fish digest (cooked, enzyme digested offal). During the period prior to 1960, raw carcasses and viscera of adult salmon included in the diet of juveniles were responsible for the complete transmission of bacterial kidney disease (Wood and Wallis, 1955) and mycobacteriosis (Ross et al., 1959; Wood and Ordal, 1958). When this practice was discontinued and pasteurized salmon parts were used in fish feed, the incidence and severity of bacterial kidney disease was reduced and mycobacteriosis was apparently eradicated from fish reared in Pacific Northwest hatcheries (Fryer and Sanders, 1981).

The pasteurized fish digest is a major protein/lipid ingredient in the fish feeds Bio-Oregon manufactures for public resource hatcheries in the Pacific Northwest. The pasteurization specifications for fish digest are set at higher standards than what is used in the dairy industry due to its slightly higher level of solids. The most comparable dairy product to the fish digest in relation to the level of solids is the ice cream mix. Both products are pasteurized by batch methods using low temperature long hold pasteurization techniques (Campbell and Marshall, 1975). The ice cream mix is held at 68.3 C for 30 minutes, whereas the fish offal is held at 65 C for 15 minutes and 82 C for 5 minutes.

Moffitt-Westover (1987) studied the bacterial flora in the Oregon Moist Pellet manufactured by Bio-Oregon. This included an examination of the pasteurized fish offal digest before and after

modifications (improvements) were made in the pasteurization process. She stated that the pasteurization specifications for the fish offal digest are sufficient for the destruction of pathogenic organisms (Moffitt-Westover, 1987). She tested the pasteurized fish digest for the presence of eight fish and nine human bacterial pathogens after process improvements were made. The fish digest was not examined for viral pathogens or Myxosporidia, specifically *Myxobolus cerebralis.* The fish pathogens included *Aeromonas hydrophila, A. salmonicida, Mycobacteria*, *Pseudomonas*, *Renibacterium salmoninarum, Vibrio anguillarum, Yersinia ruckeri*, and *Streptococcus* Group B. None of these organisms were found. *Mycobacteria, Pseudomonas*, and *Streptococcus* Group B are also human pathogens. The additional human pathogens included *Clostridium perfringens, Salmonella, Shigella, Staphylococcus aureus, Streptococcus* Group A, and *Yersinia enterocolitica.* Only *C. perfringens* was found. This is not surprising since this bacterium is widely distributed in nature and forms heat resistant spores. Therefore, if the salmon carcass analog contained some spores they would not cause significant additional exposure. Also, there are significant hurdles to the germination and growth of *C. perfringens.* This organism is a strict anaerobe and can not tolerate the level of dissolved oxygen in freshwater streams. The analog and stream lack the kind of nutrients needed for *C. perfringens* to germinate and grow, and the water temperature (3-10 C) is much colder than 37-40 C, the optimum for *C. perfringens.*

The Washington Department of Fish and Wildlife, Fish Health Laboratory, screened fishmeal that Bio-Oregon produced in the fall of 1999 from Spring Creek National Fish Hatchery fall chinook carcasses for viral and bacterial fish pathogens (Thomas, personal communication, 2000). The viral pathogens infectious hematopoietic necrosis virus (IHN), infectious pancreatic necrosis virus (IPN), and viral hemorrhagic septicemia virus (VHS) were not detected using cell culture procedures as outlined in the AFS Fish Health Bluebook. Bacteriology was done using conventional culture methods. Bacteria were only isolated in the lowest dilutions of the sample and no *Flavobacterium pyschrophilium, Flexibacter columnaris, Aeromonas salmonicida, Yersinia rucheri,* or *Vibrio sp.* were detected.

The Washington Animal Disease Diagnostic Laboratory (WADDL), Washington State University, under the direction of Danielle Stanek, DVM examined an additional eleven samples of fall chinook salmon fishmeal under a sub-contract. The nine batches made in the fall of 1999 were combined into four bulk-bags before individual batch samples were taken. Therefore, a representative fishmeal sample from each bulk-bag, and a sample of meal from each of seven batches produced in 2000, were tested for the presence of fish pathogens. These include viral, bacterial, and *M. cerebralis.* Two other fishmeal samples were examined. These include a sample of hydrolyzed marine fish fishmeal from recent production, and a sample of hydrolyzed skin/bone meal from the summer of 2000. These were produced from a mixture of pelagic marine fishes where Pacific whiting (*Merluccius productus*) predominated. These non-salmon fishmeal samples were included because these hydrolyzed meals are excellent binders, and they may be needed for that.

Following is a summary of assays performed with results for Washington Animal Disease Diagnostic Laboratory (WADDL) submission 2001-8406. The WADDL report was submitted December 4, 2001.

2001-8406: Thirteen fishmeal samples. Eleven fishmeal samples were produced from Spring Creek National Fish Hatchery fall Chinook in the fall of 1999 and 2000. Two additional samples were hydrolyzed marine fish fishmeal produced recently and hydrolyzed marine fish skin/bone meal from the summer of 2000.

Virology: All 13 processed fishmeal samples were negative for Infectious Pancreatic Necrosis Virus (IPNV), Infectious Hematopoietic Necrosis Virus (IHNV) & other filterable replicating agents. Samples were passaged twice on CHSE-214 & EPC cell lines. Positive controls of

fishmeal samples spiked with IHNV and IPNV demonstrated appropriate cytopathic effect (CPE) and fluoresced when incubated with appropriate fluorescein bound virus (either IPNV or IHNV) anti-sera. Negative control wells did not demonstrate CPE.

Bacteriology: All 13 processed fishmeal samples were cultured on Columbia blood agar (CBA) plates and found to be negative for *Aeromonas salmonicida* and *Yersinia ruckeri*. Portions of two fishmeal samples were inoculated with *A. salmonicida* and *Y. ruckeri* and streaked onto CBA plates. Isolates with biochemical characteristics consistent with either *A. salmonicida* or *Y. ruckeri* were isolated from the appropriate control plates. Positive control isolates agglutinated with commercial anti-sera for *A. salmonicida* or *Y. ruckeri*.

All fishmeal samples were also streaked onto Selective Kidney Disease Medium (SKDM) to detect the presence of *Renibacterium salmoninarum*. A portion of one fishmeal sample was inoculated with *R. salmoninarum* and streaked onto a SKDM plate to act as a positive control. No isolates morphologically and biochemically consistent with *R. salmoninarum* were isolated from any samples including the control plate during the nineteen week incubation period. Possible explanations for the failure of the positive control to grow include: inoculation of non-viable organism, culture media was unable to support growth, the dose of the control organism inoculated was inadequate, and growth of the organism was inhibited by the fishmeal. Since media produced by the same agency supported growth of *R. salmoninarum* in the past as well as for the second set of fishmeal samples (those submitted for Task 1.2), it seems likely that the cause would be one of the other three explanations.

Fishmeal samples, including a portion of one fishmeal sample and one sample diluent spiked with organism, were tested on a *R. salmoninarum* monoclonal enzyme linked immunosorbent assay (ELISA). All samples except for the spiked positive controls had a negative optical density (OD) reading. This assay has not been validated for testing fishmeal samples.

Parasitology: All 13 samples were processed by the Pepsin-Trypsin Digest assay. All samples except for the hydrolyzed marine fish fishmeal and hydrolyzed marine skin/bone meal had tailed myxospores present (possibly a *Henneguya* sp.). A few myxospores without tails were seen. These may have sustained damage causing removal of the tail during processing, tails may have been obscured by other debris on the slide (the final sample preparations contained moderate to large amounts of debris) or myxospores lacking tails may represent another myxosporean species. *Myxobolus cerebralis* myxospores do not have tails. The Pepsin-Trypsin Digest assay does not provide differentiation between viable and non-viable myxospores. In addition, this assay has not been validated for screening fishmeal samples.

Since fish health professionals in the Northwest do not agree on the level of risk associated with *Myxobolus cerebralis*, Bio-Oregon requested that the Pacific Northwest Fish Health Protection Committee (PNFHPC) establish a protocol for testing fishmeal for this myxosporidian. If this was done, it was not communicated to Bio-Oregon. The impression Bio-Oregon got talking with Ray Brunson (Olympia Fish Health Center, USFWS), PNFHPC secretary, was that Bio-Oregon just needed to satisfy the concerns of Dr. Keith Johnson (Idaho Fish & Game) because everyone else on the committee was not that concerned. Dr. Rich Holt (Oregon Fish & Wildlife) has not seen *Myxobolus cerebralis* in the lower Columbia River, including the area of Spring Creek Hatchery. Dr. Keith Johnson examined four salmon carcass analog pellets that were made from the salmon fishmeal produced by Bio-Oregon. *Myxobolus cerebralis* spores associated with whirling disease were not detected.

On January 28, 2002, Dr. Susan Gutenberger (Lower Columbia River Fish Health Center, USFWS) examined head cores from 56 Tule fall chinook salmon adults, BY01, that returned to Spring Creek Hatchery in the fall of 2001 for *Myxobolus cerebralis* spores. Results were negative for the parasite.

Task 1.2. A precise quantitative experiment was conducted to demonstrate that fish pathogens are completely inactivated during the cooking/drying process to make salmon meal.

On October 2, 2001, a precise quantitative experiment was conducted to demonstrate that selected fish pathogens are completely inactivated during the cooking/drying process to make salmon meal.

Inoculums of IHNV, IPNV, *Aeromonas salmonicida*, and *Renibacterium salmoninarum* were obtained from Craig Banner (Oregon Department of Fish and Wildlife, Oregon State University) on October 1, 2001 at 2:00 PM and placed on ice. The following information was provided with the samples.

IHNV: 25ml @ 2.6 x 10^7 PFU/ml equals 6.5 x 10^8 total PFU (plaque forming units)

IPNV: 25ml @ 2.0 x 10^9 PFU/ml equals 5.0 x 10^{10} total PFU

Aeromonas salmonicida – there were two flasks

1. *A. salmonicida* 10-1-01, LB-CT-901
2. *A. salmonicida* chinook salmon isolate RC-ChS-901, heaviest growth

Both flasks contained 140ml with concentrations of *A. salmonicida* exceeding 10^8 colony forming units (CFU)/ml.

Renibacterium salmoninarum

10-1-01, Bonn 5-Chs-LR94

Flask contained 140ml with a concentration of *R. salmoninarum* exceeding 10^{10} CFU/ml

In the early morning of October 2, 2001 fishmeal dryer number two (2) and three (3) were loaded with 3,664 and 3,988 pounds, respectively, of raw ground fall chinook salmon carcasses from Spring Creek National Fish Hatchery.

Each bacteria/virus combination was diluted with four gallons of phosphate buffered saline and mixed well for one minute. This was done to facilitate the distribution of the microorganisms in the raw ground salmon. The diluted *A. salmonicida* and IPNV cultures were added to the contents of dryer two at 8:30am. The diluted *R. salmoninarum* and IHNV cultures were added to the contents of dryer three at 9:00am. After ten minutes of mixing, approximately 500g of the salmon/bacteria-virus was removed from each dryer and placed in sterile WhirlPack bags. These were the before-heat-treatment samples of inoculated raw ground salmon. Once these samples were taken, the steam was turned on to start the cooking/drying process.

The contents of dryer two reached a moisture level of 9.02% at 8:27pm, and dryer three reached a moisture of 9.05% at 11:21pm. At these times the steam was turned off, but mixing continued until the following morning which enabled the contents to cool to about 70 F. At approximately 8:30am the contents of both dryers were removed, 755 pounds from dryer two and 996 pounds form dryer three. A representative 500g sample of fishmeal was collected from each dryer during the unloading process. These were the after-heat-treatment samples of inoculated raw ground salmon now fishmeal.

The before and after heat treatment samples of salmon carcass material and a small amount of the virus and bacterial cultures were sent to Danielle Stanek, DVM at WADDL on October 3 via UPS-Next Day Air in an insulated shipping container along with some artificial ice packs. They arrived about 2:00pm the following afternoon. The *A. salmonicida* and *R. salmoninarum* cultures were frozen but measures were taken to recover as many viable organisms as possible

Following is a summary of assays performed with results for Washington Animal Disease Diagnostic Laboratory (WADDL) submission 2001-11730.

2001-11730: Samples received were: (1) IHNV in culture media, (2) IPNV in culture media, (3) & (4) two different strains of *Aeromonas salmonicida* in culture broth, (5) *Renibacterium salmoninarum* in culture broth, (6) raw ground salmon spiked with IPNV & *A. salmonicida* (mixture of strains from samples (3) & (4), (7) raw ground salmon spiked with IHNV & *R. salmoninarum*, (8) fishmeal spiked with IPNV & *A. salmonicida*, and (9) fishmeal spiked with IHNV & *R. salmoninarum*. Samples 1-7 were positive controls; samples 8 & 9 were test samples. Positive control organisms used for WADDL case 2001-8406 were from different stocks than those used for WADDL case 2001-11730.

Virology: The IHNV in culture, the IPNV in culture, and the raw ground salmon spiked with IPNV & *A. salmonicida* (samples 1, 2 & 6) all demonstrated CPE appropriate to either IHNV or IPNV. The raw ground salmon spiked with IPNV & *A. salmonicida* (sample 6) and the IPNV in culture (sample 2) demonstrated fluorescence when incubated with IPNV fluorescein bound virus anti-sera. The IHNV in culture (sample 1) demonstrated fluorescence when incubated with IHNV fluorescein bound virus anti-sera. Samples were passaged twice on CHSE-214 & EPC cell lines. No CPE was noted in any of the other samples. Since the IHNV in culture media (sample 1) replicated in cell culture, possible reasons that the raw ground salmon spiked with IHNV & *R. salmoninarum* (sample 7) may not have demonstrated CPE are that the IHNV inoculating dose was inadequate or that the raw ground salmon itself reduced virus viability.

Bacteriology: Samples were inoculated onto CBA and SKDM plates. Isolates on CBA plates from cultures of both strains of *A. salmonicida* from broth (samples 3 & 4) were morphologically, biochemically and serologically (using anti-sera agglutination) consistent with *A. salmonicida*. The predominant isolate on SKDM plates from culture of *R. salmoninarum* from broth (sample 5) was morphologically and biochemically consistent with *R. salmoninarum.* The isolate was also positive for the *R. salmoninarum* P57 antigen using a commercial monoclonal Ellis kit. Neither *A. salmonicida* nor *R.. salmoninarum* were isolated from spiked raw ground salmon (samples 6 & 7). However, large numbers of other bacteria were present and may have overgrown the two organisms of concern. The full incubation period for *R. salmoninarum* (19 weeks) was not achieved for sample 7 due to the amount of overgrowth present. The fishmeal samples (samples 8 & 9) had low numbers of contaminating bacteria present, suggesting that heat processing (steam tube drying) substantially reduced the bacterial load present in the raw ground salmon. *Aeromonas salmonicida* was not isolated from sample 8 and *R. salmoninarum* was not detected in sample 9.

Renibacterium salmoninarum monoclonal ELISA was positive for only the *R. salmoninarum* in broth (sample 5). Since previous ELISA results were positive for fishmeal spiked with *R. salmoninarum* (WADDL case 2001-8406), possible reasons for failing to demonstrate the presence of *R. salmoninarum* in the spiked raw ground salmon (positive control) are that the kit sensitivity for raw ground salmon is less than for fishmeal, or that the inoculation dose in the raw ground salmon was at a level beneath the limits of detection for the kit. Another difference to consider is that with fishmeal the assay was run immediately after the sample was spiked, while with raw ground salmon testing occurred more than two weeks after spiking occurred.

Parasitology: Parasitology was not performed on these samples.

The results of the precise quantitative experiment to demonstrate that selected fish pathogens are completely inactivated during the cooking/drying process to make salmon meal are not conclusive. However, Dr. Stanek feels that since it was demonstrated that the process did inactivate the IPNV it would probable also knock out the INHV. Regarding the selected bacterial pathogens, Dr. Stanek feels that even though the *A. salmonicida* and *R. salmoninarum* were not isolated from the spiked raw ground salmon, (positive control) the conditions of heat processing probably would have destroyed them given the substantial reduction in the bacterial load present in the raw ground salmon when it was dried to a meal.

Task 1.3. Formulation of the salmon carcass analog to achieve desired texture and dissolution rate.

Research was needed to find an appropriate additive(s) that would; (1) effect a flesh-like texture as the analog picked up water, and (2) control the rate the analog dissolved as hydration continued. We wanted the analog to dissolve at the same rate that a salmon carcass would decompose at the expected mean water temperature. The water temperatures were expected to be unusually warm in the fall of 2001 due to low flow conditions, i.e., 20 to 10 C between mid September and the end of October. The exact size and shape of the analog was yet to be determined. The nutrient content of chinook salmon meal made from fall 1999 carcasses was used to establish the target N:P ratio of 6:1 for the salmon carcass analog.

Initial recommendations regarding the formulation and manufacturing of the salmon carcass analog came from Mr. Darrell Malczewski with Prater Industries, Cicero, IL. Our initial plan was to have Prater Industries manufacture the salmon carcass analog using compaction technology. Bio-Oregon's shop made a test pellet press from mild (A36) steel to subject the salmon meal and additives to compaction pressures of 15,000-30,000psi, which is achieved with an applied pressure of 9 to 18 tons. However, repeated application of these pressures caused the barrel of the press to expand. When this occurred, a portion of the salmon meal plus the additive was able to slip by the piston as pressure was applied. This prevented us from achieving the desired pressure, which generally resulted in a pellet fracture. Therefore, we had a local company (A. F. Dick Manufacturing) make us a new test pellet press using hardened, high carbon steel (8620), which would withstand the high compaction pressures. The test pellet press consisted of a 4-inch long barrel with O.D and I.D. of 3-inches and 1.25-inches, respectively. The piston was 5-inches long with a diameter slightly less than 1.25-inches. There was also a removable base plate with plug to seal one end of the barrel. One-third cup of the material to compact was loaded into the other end of the barrel. Once loaded, the piston was placed in the barrel and driven in to compact the material using a hydraulic press. The target pressure was held for 15 seconds before the pressure was released. After the pressure was release, the test pellet press was removed from the hydraulic press, the base plate/plug was removed, and the

formed pellet was driven out using an Arbor press. The resulting pellet or salmon carcass analog was 1.25-inches in diameter and about 1.5-inches long.

Mr. Malczewski initially recommended a pre-gelatinized corn flour to bind the salmon meal and control dissolution because it was found to be an effective additive to bind the chemical stream fertilizer used in British Columbia, Canada, and control its dissolution. This chemical is struvite, which is $MgNH_4PO_4$-HOH. The initial variables tested were: (1) compaction pressures of 9, 12, 15, and 18-tons; (2) pre-gelatinized corn flour at 3, 5, and 7%; (3) salmon meal particle size achieved with a 32-mesh screen (0.63mm opening), 50-mesh screen (0.368mm opening), and 80-mesh screen (0.224mm opening); and (4) an added water level of 3, 5, and 7%. Seven percent added water is about the maximum amount the compaction equipment used by Prater Industries can handle.

There was no apparent affect of any of these variables on the dissolution rate of the salmon carcass analogs. All conditions employed resulted in dense durable pellets. However, all the analogs dissolved in 9.4 to 10.1 hours in static water at 10 C. The salmon meal/corn flour additive mixture just sloughed off as the water penetrated the analog. There was never an appearance of softening which preceded the sloughing-off.

A number of other additives/binders were evaluated using compaction technology (our test pellet press) coupled with 50-mesh salmon meal, 7% water, and 12 tons (20,000psi) of pressure. These included: (1) wheat starch, which will gelatinize under 20,000psi of pressure; (2) a combination of two refined alginates; (3) sodium carboxymethylcellulose (CMC); (4) guar gum; (5) Pacific whiting fishmeal including partially hydrolyzed protein; and (6) bovine gelatin. None of these additives had a pronounced affect on the dissolution rate of the analog under the conditions employed. We hoped that the water entering the analog would activate one of the included binders and slow the dissolution rate of the analog. This did not occur with the binders that were tried.

At this point a decision was made to evaluate cold extrusion as a method of manufacturing the salmon carcass analog. Cold extrusion is used by Bio-Oregon to make pelleted fish feed, up to 10mm (3/8-inch) in diameter. A hydraulic motor-driven auger pushes the pellet dough (20-26% moisture) through a die plate with many holes of the desired pellet diameter. A spinning knife cuts the pellets off at the desired length, usually equal to the hole diameter. The advantage of this technology compared to compaction technology is that moisture levels up to 30% are tolerated. This enables the use of binders that require greater amounts of water, and if necessary heat, for their activation. Following extrusion, this water is removed by evaporation using a natural gas dryer. This is necessary to form a physically durable, microbiologically stable pellet.

This decision was made during July 2001, during the high period of Bio-Oregon's production of high-protein, low-ash fishmeal from Pacific whiting (*Merluccius productus*) offal. Three by-products of meal production, which could be used in the production of the salmon carcass analog, are soluble protein and oil from the stickwater (liquid removed from the cooked solids) and bone. The stickwater contains water-soluble proteins of high average molecular weight. These proteins constitute fish gelatin and are derived from collagen by the hydrolytic action that occurs when the collagen containing tissues (skin and bone) are cooked. These soluble proteins form a gel in solutions below 15-17 C. Therefore, our first tests were to determine the efficacy of this gelatin for binding the salmon carcass analog. In order to maintain the target N:P ratio of 6:1, the bone could be used to bring the level of phosphorus back up to counter act its dilution by the soluble proteins. Fish oil would be used as needed to lubricate the dough, which is necessary for cold extrusion.

As is, stickwater contains about 90% moisture. In order to get what we thought was a reasonable level of stickwater soluble protein into the salmon carcass analog, we needed to dehydrate the stickwater, i.e., make the soluble proteins more concentrated, as the stickwater's moisture level limits its level in the salmon carcass analog. The target moisture level for the salmon carcass analog dough was 30%, which was about as high as we could go and still maintain the desirable dough characteristics necessary for extrusion.

We made salmon carcass analogs with condensed stickwater on two separate occasions. On both occasions we condensed the stickwater to 65-67% moisture. At this moisture level, we were able to include about 30% in the salmon carcass analog. As such, it accounted for 10-11% of the nitrogen and phosphorus. We tried one formulation without and one with about 10% hydrolyzed Pacific whiting meal because it has had excellent binding properties in some formulations. The salmon carcass analog made with hydrolyzed Pacific whiting meal dissolved quickly in water at 5 C. The formulation without the hydrolyzed Pacific whiting meal was stable in water up to 10 C. Above that, the analog started to soften and fell apart once the temperature reached 15 C. I let some of the condensed stickwater gel on its own in the refrigerator at 5 C overnight. After it was removed from the refrigerator the following morning I observed its consistency as it warmed to room temperature. It started to soften at 12 C and was a liquid at 15 C. Since we anticipated that the water temperatures in the Yakima basin would exceed 15 C in September when the analogs would be distributed, we needed to find a binder with a higher melting point. Fish soluble protein from a warm water fish species might have worked, e.g., the melting point of tuna soluble protein is about 26 C, but that was not available.

However, we have a lot of experience with bovine gelatin at Bio-Oregon, and its melting point is 35-40 C. In our first experiment utilizing gelatin we used a combination of gelatin and stickwater, where stickwater provided the water to dissolve the gelatin. Gelatin was 5% of the gelatin/stickwater mixture. The stickwater was condensed to 77% moisture. Therefore, there was more than enough water to dissolve the gelatin. In this formulation gelatin was only 1.5% of the analog dough, and as such provided only 2.3% of the nitrogen. Salmon meal provided over 88% of the nitrogen, nearly 9% came from stickwater, and less than 1% came from wet fish bone. The dough contained 27.5% moisture, and was extruded through a 10mm (3/8-inch) die and cut to the desired length. The pellets were dried in a warm forced air natural gas dryer overnight. The dried/cooled pellets were placed in 20 C water where they dissolved in just a few hours. Therefore, 1.5% gelatin was not enough.

The next experiment was done in the laboratory to determine how much gelatin we could get into the salmon carcass analog formula. Four formulations were made wherein the gelatin was 10%, 15%, 20%, and 25% of the gelatin/stickwater mixture. The stickwater was not condensed and contained 88% moisture. The dough formula constants were 9.2% fat, 30% moisture, 8.38% nitrogen, and 1.4% phosphorus. Small amounts of marine fish oil and dried fish bone were needed to achieve these constants in the four formulations. Based on the appearance of the dough, the formulation containing the 25% gelatin/stickwater mixture probably contained close to the maximum amount of gelatin, which was a little more than 9.3%. At this level the gelatin provided the dough with 1.3% nitrogen, which was 15.5% of the total. Salmon meal provided 80% of the nitrogen. The test pellet press with 180 pounds of pressure was used to form several 1.25-inch pellets with the four formulations. These were dried overnight in a forced air convection oven at 30 C (86 F). The following morning they were placed in water at 5 C. After a few days the pellets were examined. There was a direct correlation between gelatin concentration and pellet toughness up to 20% gelatin. There was no discernable difference between those made with either 20% or 25% gelatin.

We needed to have an extruder die made with larger holes, something greater than 3/8-inch. Intuitively, we felt that a one-inch diameter salmon carcass analog would be about the right size. However, we did not know if our machinery could deal with pellets that large. Therefore, A.F. Dick Manufacturing made a die with ¾-inch and 1-inch holes. We made a salmon carcass analog dough with 20% gelatin. We put about 350 pounds of dough through the ¾-inch and the 1-inch die holes. The ¾-inch pellets extruded fine and there was no special handling needed. The 1-inch pellets plugged the extruder cone almost immediately. This cone is inverted and its function is to concentrate the pellets for delivery to a conveyor belt. We were able to solve this problem by extruding through just one, 1-inch die hole and by extruding very slow.

These pellets were dried overnight in a warm, forced air natural gas dryer. After they had cooled to the ambient air temperature they were placed in 20 C water. In a few days they had softened, but were still intact. After about three weeks they had softened a little more, but were still intact, and they were covered with mold. Also, the 1-inch pellets were in a little better shape than the ¾-inch pellets. At this time we needed to make a decision regarding the formulation and size of the salmon carcass analogs which would be distributed into Yakima River tributaries in a couple weeks. To be on the safe side we decided to make them 1-inch in diameter and close to that length, and with 25% gelatin.

Task 1.4. Production of the salmon carcass analog. Analogs destined for placement in tributaries of the Yakima and Klickitat Rivers were made on two occasions.

On September 7, 2001 4,246 pounds of salmon carcass analog dough was made with the following composition. It contained 65.36% salmon meal, 23.55% hydrolyzed, pasteurized, and deboned marine fish offal, 7.84% gelatin, 2.31% dried marine fish bone, and 0.94% Algibind. Algibind is a crude sodium alginate manufactured from seaweed, specifically *Ascophyllum nodosum*. The initial mix was a little goopy/oily. Algibind was added to help tie up the oil, and we dried up the mix a little by adding about 2% more of the ground salmon meal/fish bone blend than the target formula called for. The salmon meal at 7.5% moisture contained 11.42% nitrogen and 1.9% phosphorus. We were trying to come as close to those values as possible for the salmon carcass analog. The target formula for the salmon carcass analog would have resulted in a product (at 12% moisture) with 10.75% nitrogen and 1.79% phosphorus and a N:P ratio of 6:1. The changes in the mixer that were necessary to achieve a dough with desirable physical characteristics resulted in a salmon carcass analog with 10.73% nitrogen and 1.89% phosphorus, which reduced the N:P ratio from the target 6:1 to 5.68:1.

The adjusted dough extruded fine. The 1-inch salmon carcass analog pellets were dried for close to three days using forced ambient temperature air. We dried the analogs without heat to maximize their density. The salmon carcass analogs were removed from the dryer and screened to remove overs and unders. We produced 57 boxes of 20 kilograms (44 pounds) each or close to 2,514 pounds of salmon carcass analogs ready for distribution into the Yakima River treatment tributaries.

On August 30, 2002, 6,000 pounds of salmon carcass analog dough was made with the following composition. It contained 46.41% salmon meal, 16.95% whole Pacific sardine (*Sardinops sagax* Jenyns)/salmon scrap meal, 24.63% hydrolyzed, pasteurized, and deboned marine fish offal, 8.21% gelatin, and 3.80% dried marine fish bone. The salmon meal used was produced from Spring Creek National Fish Hatchery Fall Chinook that were sacrificed during the fall of 2001. Most of these had not been spawned, but were killed soon after entering the hatchery. This occurred because many

more adults returned to the hatchery than were needed for production purposes. Because of this, the lipid level of the carcasses and resulting meal was much higher than the meal we used last September to make the analogs, 17% and 9.6%, respectively. This high level of lipid prevented us from grinding the meal alone. It was necessary to blend the salmon meal with some deboned/deoiled whole Pacific sardine/salmon scrap meal and dried marine fish bone to bring the lipid level of this mixture down to 14%. It was necessary to add a small amount of bone in order to achieve the nitrogen:phosphorus ratio of 6:1. The formula for the salmon carcass analogs should result in a product (at 3% moisture) with 11.77% nitrogen and 1.96% phosphorus and a N:P ratio of 6:1.

The meal blend was difficult to grind at 14% lipid. In the future it will need to be lower. The dough extruded with difficulty because it was very tough. The 1-inch salmon carcass analog pellets were dried for about one hour using forced 200 F temperature air. Prior experimentation suggested that it was not necessary to dry the analogs with cooler air in order to achieve maximum density. The salmon carcass analogs were removed from the dryer and screened to remove overs and unders. We produced 91 boxes of 20 kilograms (44 pounds) each or close to 4,000 pounds of salmon carcass analogs ready for distribution into the Yakima and Klickitat River treatment tributaries.

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